

Session 8: Mitochondria in Ischemia-Reperfusion and Cardiomyopathy

8-01. The metabolic activators of mitochondrial ATP-dependent potassium channel and its role in cardioprotection.

Galina D Mironova¹, IB Krylova², AE Negoda¹, OM Rodionova², EV Kachaeva¹, NP Evdokimova², NS Sapronov²

¹Inst. Theoretical Experimental Biophysics, Russian Acad. Sci., Pushchino, Moscow region, 142290 Russia; ²Inst. Experimental Medicine, Russian Acad. Medical Sciences, St. Petersburg, Russia. - [mironova@iteb.ru](mailto:ironova@iteb.ru)

Cardiac disorders are a common cause of death in technically developed countries, so the search for principally new ways of their prevention and treatment is a problem of great concern. A development of new approaches based on the data of basic studies obtained lately is important in this perspective. It is well known that synthetic activators of the mitochondrial ATP-dependent K⁺ channel (mitoK_{ATP}) have a clear cardioprotecting properties and prevented ATP disintegration in experimental myocardial ischemia [1]. In our earlier research we found that uridine-5'-diphosphate (UDP) is a physiological activator of the mitoK_{ATP} [2].

The goal of this study is to investigate a possible role of metabolic activators of mitoK_{ATP} in cardioprotection. We studied the cardioprotective properties of the precursors of UDP, uridine and uridine-5'-monophosphate (UMP). These precursors opposed to UDP possess the capacity to penetrate into the cell. On a model of acute myocardial infarction in rats the anti-ischemic activity of these preparations was revealed. It was shown that the myocardium infarct zone decreased in 1.9 and 3.5 times for uridine and UMP, respectively. The changes of the T-wave amplitude, electrophysiological characteristic of myocardium injuries during infarction, confirmed these data. The results obtained showed the same trend of changes of the T-wave amplitude under the treatment of the preparations. The inhibitors of mitoK_{ATP} glibenclamide and 5-hydroxydecanoic acid (5-HD) prevented the cardioprotective effect of uridine and UMP. This suggests that mitoK_{ATP} is involved in the realization of the anti-ischemic effect of uridine and its phosphonucleotide. It was also found that uridine and UMP affect the development of occlusion arrhythmias in rats, the effect of UMP being more pronounced. Since the antiarrhythmic action of 5-HD was less manifested, than the effect of glibenclamide we suppose that their antiarrhythmic action is associated mainly with the activation of the cell membrane ATP-dependent potassium channel (cellK_{ATP}). Thus both preparations can be considered as potential cardiotropic agents, but the mechanism of their action can be different.

Supported by Russia Foundation of Basic Research grant No/ 04-04-97281.

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8-02. Effects of NO and estrogens on ischaemia-induced permeability transition in heart mitochondria.

Vilma Borutaite¹, R Morkuniene², G Brown¹

¹Dept. Biochemistry, Univ. Cambridge, Cambridge, UK; ²Kaunas Univ. Medicine, Kaunas, Lithuania. - vb207@mole.bio.cam.ac.uk

Apoptosis is known to contribute to ischaemia-reperfusion induced cell death in the heart. However, the mechanism of induction of apoptosis during ischaemia or reperfusion is still unclear. Recently we have shown that global ischaemia by itself (without reperfusion) can induce cytochrome c release from mitochondria and subsequent apoptosis through opening of mitochondrial permeability transition pore (MPT) [1]. In this study we aimed to investigate whether protective action of nitric oxide (NO) and estradiol, two well known cardioprotective agents, can be associated with their effect on MPT during heart ischemia.

NO depending on concentrations and other conditions can be cytotoxic or cytoprotective. For example, S-nitrosothiols can rapidly induce mitochondria-mediated apoptosis in the perfused heart [2]. In contrast, NO itself at relatively low concentrations can be cardioprotective. We found that short (3-5 min) pre-perfusion of hearts with micromolar concentrations of NO donor DETA/NO protected hearts from ischemia-induced cytochrome c release from mitochondria and subsequent respiratory inhibition and caspase activation. Similarly, perfusion of the hearts with 100 nM estradiol prevented the loss of cytochrome c from mitochondria and its accumulation in the cytosol as well as inhibition of mitochondrial respiration, caspase activation and nuclear apoptosis. In isolated mitochondria, estradiol prevented MPT related, high calcium induced loss of cytochrome. These data suggest that estrogens and NO can protect the myocardium against ischemia-induced apoptosis by inhibiting MPT.

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8-03. Role of matrix calcium accumulation in mitochondrial permeability transition in postconditioned versus preconditioned myocardium.

Odile Gateau-Roesch, L Argaud, J Loufouat, E Couture-Lepetit, J Adobati, M Ovize

INSERM E0226 "Cardioprotection" - Lab. Physiologie Lyon-Nord / Université Claude Bernard Lyon1 - Hospices Civils de Lyon, France. - roesch@rockefeller.univ-lyon1.fr

Calcium is a major trigger of mitochondrial permeability transition. Inhibition of the mitochondrial permeability transition pore (mPTP) opening plays a key role in cardioprotection afforded by both postconditioning (PostC) and preconditioning (PreC). We investigated whether inhibition of mPTP in PostC and PreC might be related to a change in mitochondrial calcium after ischemia-reperfusion.

Anesthetized NZW rabbits ($n=9$ /group) underwent either no intervention (sham) or 30 min of ischemia (I) followed by 1 h of reperfusion R. Animals underwent either no intervention (control, C), PreC by 5 min ischemia / 5 min reperfusion, or PostC with 4 episodes of 1 min ischemia / 1 min reperfusion performed after 1 min of reperfusion after the 30 min ischemia. At the end of the protocol, area at risk mitochondria were isolated by differential centrifugations. Total mitochondrial calcium content was assessed after mineralisation by HNO₃ and measured by Induced Coupled Plasma Atomic Emission Spectrometry (ICP AES). Using freeze/thawed detergent-treated mitochondria, ionized (free) mitochondrial calcium content was measured using a calibrated Ca²⁺-selective microelectrode. We also assessed the Ca²⁺ resistance capacity CRC of mPTP defined as

the calcium overload required to induce mPTP opening. Moreover we checked mitochondrial integrity by electron microscopy.

In controls, CRC was significantly reduced with Ca^{2+} overload required for mPTP opening averaging 0.73 ± 0.16 μg calcium/mg of mitochondrial proteins versus 4.23 ± 0.17 in sham hearts ($P < 0.0001$). PostC, as PreC attenuated CRC reduction with Ca^{2+} overload averaging 1.58 ± 0.14 and 1.91 ± 0.26 μg calcium/mg prot. respectively ($P < 0.005$ versus C). When compared to shams (1.42 ± 0.09), total mitochondrial Ca^{2+} content was significantly increased in both controls (2.39 ± 0.43 μg calcium/mg prot.) and PostC (2.34 ± 0.37), but not in PreC group (1.29 ± 0.17). Similar patterns were obtained for ionized mitochondrial Ca^{2+} content with a significant difference ($P < 0.001$) versus sham (0.16 ± 0.01 μg calcium/mg Prot.) in controls (0.61 ± 0.10) and PostC group (0.77 ± 0.15), but not in PreC (0.26 ± 0.05). Electron photomicrograph clearly showed a lot of swollen mitochondria with damaged cristae in control group myocardium, while many mitochondria in PreC and particularly in PostC group displayed intact membranes and dense matrix.

These data suggest that restoration of mitochondria integrity play a major role in cardioprotection by PreC and PostC treatment. Mitochondrial permeability transition may be regulated in different ways in postconditioning versus preconditioning since reduced susceptibility to calcium overload observed in PreC and PostC were not correlated with the same repartition of mitochondrial calcium in these 2 groups.

8-04. Mitochondrial function in cardiac ischemia-reperfusion injury and ischemic preconditioning.

Paul S Brookes

Dept. Anesthesiology, Univ. Rochester Medical Center, Rochester, NY 14642, USA. - paul_brookes@urmc.rochester.edu

Mitochondrial dysfunction has long been recognized as a key event in cardiac ischemia-reperfusion (I-R) injury. In addition recent evidence has invoked a role for mitochondria in ischemic preconditioning (IPC). Several aspects of mitochondrial function have been investigated in both I-R and IPC, including: (i) the proton permeability (H^+) leak of the inner membrane, (ii) post-translational modifications to complex I, (iii) the role of nitric oxide (NO^*) and its redox cohorts, and (iv) the regulation of ROS generation by O_2 tension and NO^* . To accomplish this, several unique methodologies have been combined, including open-flow respirometry [1], proteomics, chemiluminescent NO^* detection and novel mitochondrially targeted drugs.

Results are summarized as follows: (i) H^+ leak is reversibly elevated in IPC via a UCP dependent mechanism, and is further elevated irreversibly in I-R by a mechanism involving AMP stimulation of the ANT, and transient PT pore opening. (ii) Thiols in the 75kDa subunit of complex I are damaged in I-R, and this leads to ROS generation without significant loss of enzymatic activity. (iii) Complex I can be S-nitrosated on specific subunits under various conditions. (iv) ROS generation by mitochondria does not increase under hypoxia, but actually decreases with O_2 tension. The ramifications of these findings for therapeutic intervention in I-R injury, in particular the use of mitochondrially-targeted antioxidants, will be discussed.

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8-05. Reversible redox dependent modulation of mitochondrial aconitase and proteolytic activity status during cardiac ischemia reperfusion.

Anne-Laure Bulteau¹, L Szweda LI², B Friguet

¹Universite Paris7-Denis Diderot, Paris, France; ²Oklahoma Research Foundation, Omaha, USA. - anne-laure.bulteau@paris7.jussieu.fr

Aconitase, a citric acid cycle enzyme that converts citrate to isocitrate, belongs to the family of iron-sulfur containing dehydratases whose activities depend on the redox state of the cubane [4Fe-4S] cluster. Recent evidence indicates that mitochondrial aconitase can be reversibly inhibited or progress to irreversible inactivation and degradation in response to pro-oxidants [1]. Cardiac ischemia/reperfusion is associated with an increase in mitochondrial free radical production. In the current study, the effects of reperfusion-induced production of pro-oxidants on mitochondrial aconitase and proteolytic activity both in cytoplasm and mitochondria were determined to assess whether alterations represented a regulated response to changes in redox status or oxidative damage. Evidence is provided that ATP-dependent proteolytic activity in the mitochondria increases during early reperfusion followed by a time-dependent reduction in activity to control levels. These alterations in proteolytic activity parallel an increase and subsequent decrease in the level of oxidatively modified protein. However, proteasome activity is decreased upon the same times of reperfusion. Aconitase activity exhibited a marked decline in activity followed by reactivation during cardiac reperfusion. Loss and regain in activity involves reversible sulfhydryl modification. Aconitase was found to associate with the iron binding protein frataxin exclusively during reperfusion. *In vitro* frataxin has been shown to act as a chaperone protein that protects aconitase from [4Fe-4S]²⁺ cluster disassembly, irreversible inactivation, and potentially degradation [2]. Thus, the response of mitochondrial aconitase and ATP-dependent proteolytic activity to reperfusion-induced pro-oxidant production appears to be a regulated event that would be expected to reduce irreparable damage to the mitochondria.

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8-06. Inhibition of electron transport during ischemia prevents mitochondrial ischemic damage and protects the heart.

Q Chen, Charles L Hoppel, EJ Lesnfsky

Case Western Reserve Univ. and Louis Stokes VA Medical Center, Cleveland, OH, USA. - clh5@cwru.edu

Mitochondrial dysfunction contributes to myocardial injury during ischemia (ISC) and reperfusion (REP). ISC damages the electron transport chain leading to a decrease in the rate of oxidative phosphorylation (OXPHOS). Reversible blockade of electron transport with amytal immediately before ISC attenuates ischemic damage to OXPHOS. We proposed that protection of OXPHOS during ISC will preserve OXPHOS and decrease myocardial damage during REP.

Langendorff perfused rat hearts were treated with amytal (2.5 mM bolus for 1 min immediately before ISC) or vehicle and underwent 25 min global ISC (37 °C) and 30 min REP without additional treatment. Subsarcolemmal (SSM) and interfibrillar (IFM) mitochondria were isolated at end of REP to measure OXPHOS with glutamate as substrate. Left ventricular developed pressure (LVDP), diastolic pressure (DP) and lactate dehydrogenase (LDH) release were measured. Amytal pretreatment protected OXPHOS in SSM and IFM following ISC-REP with preserved state 3 and the decreased state 4 rates leading to improved respiratory control ratio (RCR). Amytal also prevented ischemic contracture (DP-ISC) and improved functional recovery during REP with increased left ventricular developed pressure (LVDP-REP) and decreased diastolic pressure (DP-REP). Amytal attenuated LDH release during REP and myocardial infarct size, indicating

decreased myocyte cell death. Thus, reversible blockade of electron transport during ISC preserves mitochondrial OXPHOS and mitigates myocardial damage during REP.

OXPHOS during REP [nAO·min ⁻¹ ·mg ⁻¹]	SSM State 3	SSM State 4	SSM RCR	IFM State 3	IFM State 4	IFM RCR
ISC-Rep (n=12)	122 ± 6	57 ± 4	2.3 ± 0.2	173 ± 10	74 ± 5	2.5 ± 0.2
Amytal+ISC-Rep (n=11)	172 ± 9*	35 ± 4*	5.4 ± 0.5*	274 ± 17*	43 ± 3*	6.6 ± 0.6*

Cardiac function	DP-ISC [mmHg]	LVDP-REP [mmHg]	DP-REP [mmHg]	LDH [mU·min ⁻¹ ·g ⁻¹]
ISC-REP (n=12)	54 ± 6	57 ± 4	37 ± 4	427 ± 60 (n=11)
Amytal+ISC-REP (n=11)	5 ± 1*	79 ± 5*	2 ± 1*	273 ± 28* (n=9)

Mean ± SEM. * P<0.05 vs. ISC+REP

8-07. Does endurance training limit rat heart mitochondrial dysfunction induced by *in vitro* anoxia-reoxygenation?

António Ascensão¹, J Magalhães¹, JMC Soares¹, R Ferreira¹, MJ Neuparth¹,
F Marques^{3,4}, PJ Oliveira^{5,6}, JA Duarte^{1,2}

¹Dept. Sport Biology; ²Center Research in Physical Activity and Leisure, Fac. Sport Sciences; ³Dept. Biochemistry Clinical Analysis, Fac. Pharmacy; ⁴Inst. Molecular Cell Biology, Univ. Porto; ⁵Dept. Zoology; ⁶Centre Neurosciences Cell Biology, Univ. Coimbra, Portugal. - aascensao@fcdef.up.pt

Mitochondria are clearly involved in the physiopathology of many cardiac dysfunctions [3] and endurance training is known to improve the tolerance of the heart and specifically of heart mitochondria to *in vivo* oxidative-based insults [1,2]. However, the effect of endurance training on heart mitochondrial function submitted to *in vitro* anoxia-reoxygenation (A-R) is poorly understood. The present work intended to analyse the effect of moderate endurance treadmill training (14-wk) against rat heart mitochondrial dysfunction induced by *in vitro* A-R.

The respiratory parameters state 3, state 4, ADP/O and respiratory control ratio- RCR, as well as biochemical markers of protein oxidation (carbonyl groups) and lipid peroxidation (malondialdehyde) were determined in isolated mitochondria before and after 1 min anoxia followed by 4 min reoxygenation. Basal levels of heat shock protein 60 kDa (HSP60) and 70 kDa (HSP70) were measured in mitochondria and whole muscle homogenate, respectively.

A-R significantly impaired the rate of state 3 and state 4 respiration, as well as the RCR and ADP/O in the sedentary group. Nevertheless, mitochondrial state 3 respiration was significantly higher in trained than in the non-trained group both before and after A-R. The impairments in RCR, ADP/O ratio and state 4 induced by A-R in non-trained group were significantly attenuated in endurance-trained group. Oxidative modifications of mitochondrial proteins and phospholipids were found in sedentary group after A-R, although limited in trained group. Increased levels of mitochondrial HSP60 and tissue HSP70 accompanied the lower decrease in the respiratory function after A-R observed in trained group.

It is concluded that previous 14-wk endurance training limited the impairments on rat heart mitochondria caused by *in vitro* A-R.

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8-08. Mitochondrial mechanism of stress-induced cardiomyocyte injury.

Lingjia Qian, H Ren, J Gong, W Wang

Inst. Health Environmental Medicine, Tianjin 300050, China. - qianlj2000@hotmail.com

It is confirmed that stress induces cardiovascular diseases with cardiomyocyte injury, but its cellular and molecular mechanism remains unclear. In the present study the changes of cardiac mitochondria of stressed rats were characterized to explore the influence of stress on mitochondrial function and its role in cardiomyocyte injury. The results showed that stress induced the increase of the respiratory control rate (RCR) and oxidative phosphorylation efficiency (P/O) in the mitochondria of cardiomyocytes in the time and dose dependent fashion, and resulted in the disorder of intracellular Ca^{2+} balance. The stress induced alteration of mitochondrial membrane permeability transition (MPT) was also found, which in turn led to the release of cytochrome c from mitochondria to cytosol and activated caspase cascade in cardiomyocytes. Stress also increased the Fas expression and activated the Fas pathway through acting on the mitochondrial MPT. Mechanism. Bcl-2 overexpression in cardiomyocyte protected the mitochondria from stress injury and reduced the cardiomyocyte death, including apoptosis and necrosis, induced by stress. Hsp70 also depressed the Fas-mitochondria pathway to decrease the death rate of cardiomyocyte under stress loading. As a conclusion it appears that the mitochondria play an important role in the mechanism of stress induced cardiomyocyte injury. Both Bcl-2 and Hsp70 seem the key regulators in the mitochondrial mechanism of stress induced cardiomyocyte apoptosis. These findings imply the possibility that regulating the expression of Bcl-2 or Hsp70 to protect mitochondria acts as a new therapeutic principle in cardiovascular diseases.

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8-09A. Mitochondrial dysfunction and oxidative stress in chagasic cardiomyopathy.

Nisha Garg

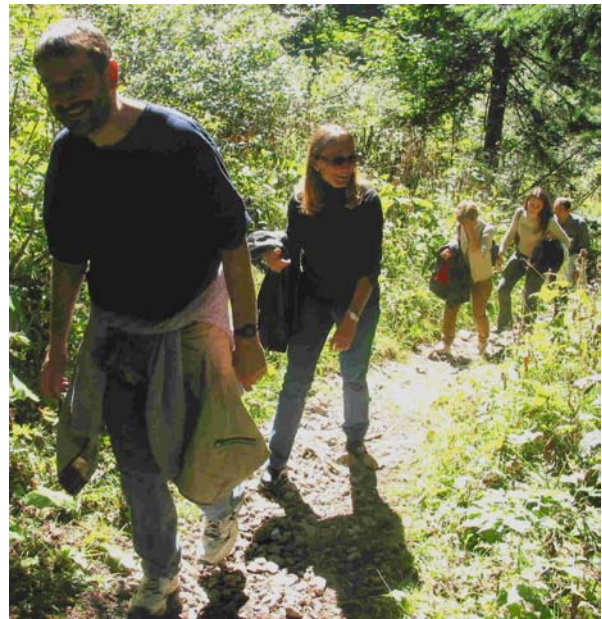
Depts. Microbiology, Immunology and Pathology, Univ. Texas Medical Branch, Galveston TX, USA. - nigarg@utmb.edu

Chagasic cardiomyopathy (CCM), is a major public health threat in Latin America and Mexico, and is recognized as an emerging infectious disease in the U.S. Endomyocardial biopsies from patients in different clinical stages of the disease have suggested that myocardial inflammation and fibrosis play an important role in the pathogenesis of CCM. Because only a few, if any, parasites are detected during progressive CCM, other factors are believed to be involved in activation and/or sustenance of the inflammatory response. These factors are, however, not known.

Our recent studies have provided a new framework for understanding the initiation and progression of CCM. We have shown in experimental models that infection by *Trypanosoma cruzi* elicits mitochondrial dysfunction that is associated with oxidative modifications and altered activities of the respiratory chain complexes, generation of reactive oxygen species (ROS), and antioxidant/oxidant imbalance in the heart. Further, we have shown that scavenging of ROS diminishes the mitochondrial dysfunction-induced oxidative stress and, subsequently, is effective in limiting the inflammatory responses,



Alpine walks and talks – Top: Susanne Arnold, Sebastian Vogt, Thierry Letellier, Hans Nohl, Eveline Hütter, Katrin Staniek.

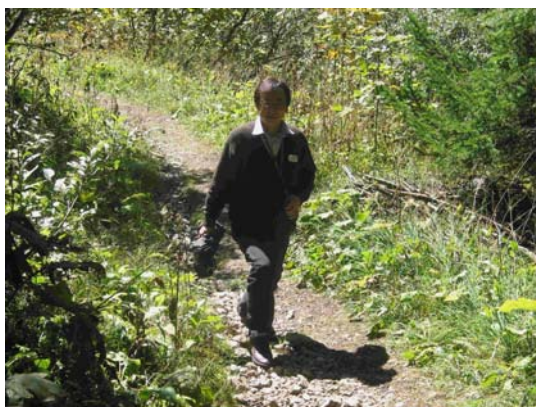


Top: Chris Cooper, Cecilia Giulivi, Daniela Curti, Barbara Santoro, Michael Verkhovsky



Top: Benjamin Faustin, Rodrigue Rossignol, Anna-Maria Aleardi, Joan McEwen, Pavel Golik, Frédéric Bouillaud.

Right: Julian Pakay, Franz Hartner, Nazzareno Capitanio.



Top: Bernhard Kadenbach, Benjamin Faustin, Thierry Letellier, Hans Nohl, Katrin Staniek, Eveline Hütter. Left: Eiji Takahashi.

Alpine walks and talks at MiP2003



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